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Responses of Early Life History Stages of the Striped Bass, 'Morone saxatilis' to Chlorination

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16 ABSTRACT

The toxicity of total residual chlorination (TRC) to early life stages of the striped bass, Morone saxatilis, was determined using percent embryo hatchability, incipient LC50 bioassays, histopathology, and avoidance responses. Beginning 8 to 9 hours after fertilization, developing embryos were exposed continuously to TRC in flowing water at 1.0-3.0 ppt salinity and 18 ± 1 C. Fifty-six percent of the control group (no TRC exposure) hatched. None of the embryos exposed to a measured TRC concentration of 0.21 mg/l hatched. Only 3.5 percent of the embryos exposed to 0.07 mg/l TRC and 23% of those exposed to 0.01 mg/l hatched. Incipient LC50 bioassays were used to determine sensitivity of 2-, 12- and 30-day-old striped bass to concentrations of TRC in flowing water (1.0-3.0 ppt salinity at 18±1 C). The estimated incipient LC50 was 0.04 mg/l TRC for 2-day-old prolarvae, 0.07 mg/l for 12-day-old larvae and 0.04 mg/l for 30-day-old juveniles. Histological examination of 30-day-old juveniles which survived exposure in the incipient LC50 bioassay indicated gill and pseudobranch damage for fish exposed to 0.21 to 2.36 mg/l TRC. Statistical analysis of avoidance tests conducted at 1.0-3.0ppt salinity and 18±1 C with 25-day-old larvae showed significant (X^2, $p < 0.05$) and reproducible avoidance responses to measured TRC concentrations of 0.79-0.82 mg/l and 0.29-0.32 mg/l. No avoidance was indicated at TRC concentrations of 0.16-0.18 mg/l.

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Responses of Early Life History Stages of the Striped Bass, *Morone saxatilis* to Chlorination[1]

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ABSTRACT: The toxicity of total residual chlorination (TRC) to early life stages of the striped bass, *Morone saxatilis*, was determined using percent embryo hatchability, incipient LC50 bioassays, histopathology, and avoidance responses.

Beginning 8 to 9 hours after fertilization, developing embryos were exposed continuously to TRC in flowing water at 1.0–3.0 ppt salinity and 18 ± 1°C. Fifty-six percent of the control group (no TRC exposure) hatched. None of the embryos exposed to a measured TRC concentration of 0.31 mg/l hatched. Only 3.8 percent of the embryos exposed to 0.07 mg/l TRC and 33 percent of those exposed to 0.01 mg/l TRC hatched.

Incipient LC50 bioassays were used to determine the sensitivity of 2-, 12- and 30-day-old striped bass to concentrations of TRC in flowing water (1.0–3.0 ppt salinity at 18 ± 1°C). The estimated incipient LC50 was 0.04 mg/l TRC for 2-day-old prolarvae, 0.07 mg/l for 12-day-old larvae and 0.04 mg/l for 30-day-old juveniles.

Histological examination of 30-day-old juveniles which survived exposure in the incipient LC50 bioassay indicated gill and pseudobranch damage for fish exposed to 0.31 to 1.36 mg/l TRC.

Statistical analysis of avoidance tests conducted at 1.0–3.0 ppt salinity and 18 ± 1°C with 24-day-old larvae showed significant (X^2, $p < 0.05$) and reproducible avoidance responses to measured TRC concentrations of 0.79–0.82 mg/l and 0.29–0.33 mg/l. No avoidance was indicated at TRC concentrations of 0.16–0.18 mg/l.

Introduction

Along the East coast of North America, the anadromous striped bass, *Morone saxatilis*, ranges from the lower St. Lawrence River, Canada, to the St. John's River, Florida. In the Gulf of Mexico, the striped bass occurs from western Florida to Louisiana (Raney 1952; Bigelow and Schroeder 1953).

Striped bass generally are found in estuarine and near-shore environments. They seldom range further than 4 to 6 km offshore (Bigelow and Schroeder 1953). Striped bass spawn in the spring and early summer depending on the latitude (Raney 1952). Spawning adults seek a river system where the currents are strong enough to prevent

[1] Gulf Breeze Contribution Number 304

the demersal eggs from settling to the bottom where they could be smothered by silt. Tresselt (1952) and Dovel (1967, 1971) determined that striped bass eggs were found within the first 40 km of freshwater in Virginia rivers. Similar observations of preferred spawning habitats were made 160 km upstream from tidal influence in the Roanoke River at Weldon, North Carolina (Worth 1903; Pearson 1938).

Ambient water temperatures at the time of spawning range from 14 to 21 C (Worth 1884; Pearson 1938). Temperatures of 15 to 19 C are optimal for survival of the developing eggs (Albrecht 1964; Bayless 1972). Prolarvae emerge 42 to 74 hours after fertilization at optimal rearing temperatures (Merriman 1941; Bigelow and Schroeder 1953).

Female striped bass reach sexual maturity in 4 to 6 years and males in 2 to 4 years (Merriman 1941; Vladykov and Wallace 1938).

Since the striped bass spends its entire life in near-shore, estuarine, or freshwater environments, the discharge of chlorinated effluents into coastal areas may adversely affect all life history stages of this species. For example, Meldrin et al. (1974) determined that another Serranidae, the white perch, *Morone americana*, (140–160 mm TL) showed avoidance responses to measured chlorine concentrations as low as 0.02 mg/l. In addition, Tsai (1970) suggested that chlorinated sewage treatment wastes discharged into the Little Patuxent River, Maryland, may block upstream spawning migrations of the white perch and other semianadromous fish.

Our study, conducted with recently spawned eggs, young larvae, and juvenile striped bass was designed to detect sensitivity of these stages to TRC in brackish water. Flowing water bioassays, histopathology, and avoidance of TRC were used to assess sensitivity.

Material and Methods

Fish Acquisition

Fertilized (water-hardened) embryos of the striped bass, *Morone saxatilis*, were obtained from the South Carolina Wildlife and Marine Resources Department, Moncks Corner Fish Hatchery, on 27 March 1975. The embryos (approximately 6 hours old) were placed in a plastic-lined styrofoam cooler for transport to the laboratory. The water temperature at the hatchery was 19 C and ranged from 19 to 20 C during embryo transport. Upon arrival at the laboratory, the embryos were placed in hatchery jars (Midland Plastics, MPC-3001)[a] at 18 ± 1 C.

Developing Embryo Bioassays

For bioassays with developing embryos, fourteen, 9-ml aliquots of a fertilized embryo-water slurry were gently pipetted into five separate hatchery jars. Each of the hatchery jars (total volume 7 liters) received a flow of 60 liters per hour of unfiltered brackish water, 1.0 to 3.0 parts per thousand (ppt) salinity. The water temperature was maintained at 18 ± 1 C with a Frigid® titanium coil water chiller and 2000 watt quartz immersion heater. A subsample consisting of six, 9-ml aliquots was taken while eggs were being distributed into respective hatchery jars. These subsamples were used to estimate the total number of embryos placed in each hatchery jar. The number of embryos per 9-ml aliquot ranged from 475 to 721, $\bar{X}$ = 603, S.D. = 100.

A range of concentrations of chlorine (Mallinckrodt, NaOCl, Analytical Reagent, minimum 5 percent chlorine) was injected into four hatchery jar bioassay systems with Sage® Model 355 Syringe Pumps (Fig 1). The fifth (untreated) hatchery jar was used as a control.

A 7.5 cm I.D. × 8.0 cm deep acrylic plastic "catch cup" with a 600 μm mesh nylon plankton netting bottom was suspended immediately below the lip of the overflow from each hatchery jar to collect non-viable embryos during the tests. Non-viable striped bass embryos float to the surface and spill from the hatchery jar (Bayless, pers. commun., Dennis Wildlife Center, Moncks Corner, S.C. 29461).

Embryos were periodically removed from each of the cups and were counted. An effort was also made to determine the stage at which development had ceased. The cups

[a] Mention of trade names does not imply endorsement by the U. S. Environmental Protection Agency.

Fig. 1. Flowing water bioassay system used to expose developing striped bass embryos to total residual chlorination. A. Temperature-controlled brackish water supply (60 l/hr). B. Hatchery jar. C. Catch cup. D. Holding aquarium (40 l). E. Standpipe screen. F. Standpipe drain. G. Infusion tube. H. Syringe pump. I. Striped bass eggs.

were removed from the hatchery jar bioassay system as soon as larvae began to hatch.

A subsample of 10 embryos was examined at the beginning of chlorine injection to confirm the developmental stage. The embryos (8 to 9 hours old) were in the late blastula-early gastrula stage of development (Bayless 1972).

Larval Bioassays

Incipient LC50 bioassays (Sprague 1964, 1969) were conducted to determine the sensitivity of 2-day-old prolarvae (4.0-4.6 mm TL, $\bar{X}$ = 4.3, S.D. = 0.20), 12-day-old larvae (5.4-8.1 mm TL, $\bar{X}$ = 6.7, S.D. = 0.9), and 30-day-old juveniles (11.9-16.0 mm TL, $\bar{X}$ = 13.6, S.D. = 1.5) to total residual chlorination.

For each test, 20 individuals were placed in each of 7 acrylic plastic flow-through aquaria, 27 cm × 15 cm × 8.7 cm (volume 6 liters), which received 18 liters of brackish water per hour. Perforated acrylic plastic baffles covered with 0.5 mm mesh nylon screen were located at the front and rear of each treatment aquaria. One baffle formed a chlorine "mixing chamber" at the point of chlorine addition to the flow-through tanks, the other prevented loss of fish through the standpipe drain. The water temperature was maintained at 18 ± 1 C for each of the incipient LC50 bioassays.

Since striped bass do not begin to feed until approximately 5 to 7 days after hatching, prolarvae were not fed during their test. Striped bass used in the incipient LC50 bioassays beginning 12 and 30 days after hatching, as well as those used in avoidance tests, were fed *Artemia* sp. nauplii twice daily, from the sixth day after hatching until tests were terminated.

Observations for time-to-death were made continuously for the high exposure concentrations of TRC and at 2- to 8-hour intervals for low exposure concentrations. Incipient LC50 tests were terminated after 2 days of exposure. Fluorescent "cool white" lamps suspended 150 cm above the bioassay aquaria provided illumination (12L:12D photoperiod, 1600 lux).

Histology

Juvenile striped bass surviving the incipient LC50 bioassay that began 30 days after hatching were removed from their respective treatment and control aquaria. Juveniles exposed to the high concentrations of TRC, 2.14 to 0.17 mg/l, were removed when 50 percent mortality was observed for respective exposure levels. Those exposed to lower TRC concentrations of 0.08 to 0.02 mg/l (no mortalities) were sacrificed after 2 days of exposure. Fish were placed in Davidson's Fixative for 24 hours, were transferred to 50 percent ETOH for 2 hours, and finally were deposited in 70 percent ETOH for storage. Striped bass were dehydrated, infiltrated with paraffin, and paraffin-embedded for sectioning sagittally and parasagittally at 7 µm. Sections were stained with Harris hematoxylin and eosin and were mounted for light microscopy.

Avoidance Tests

An avoidance apparatus similar to one described by Sprague (1968) was used to determine the response of 24-day-old striped bass larvae to TRC in flowing brackish water (1-3 ppt salinity). Tests were conducted at 18 ± 1 C.

The avoidance apparatus was constructed of clear acrylic plastic (Fig 2). Eighteen liters of water per hour was siphoned from the head trough into each mixing box. Twenty striped bass larvae were placed in

Fig. 1. Avoidance apparatus used for determining the response of 30-day old striped bass larvae to TRC in flowing brackish water. A. Temperature controlled water supply. B. Head trough. C. Standpipe overflow for maintaining constant head. D. Calibrated 18 l/hr syphons. E. Brackish water-chlorine mixing box. F. Fish entrance; chlorine-sampling ports. G. Interface drain. H. Infusion tube. I. Syringe pump. Direction of water flow indicated by arrows.

the avoidance apparatus through the fish-entrance; chlorine-sampling ports (10 through each port) adjacent to the center interface drain. The flow of 14 liters of water per hour into each side of the avoidance chamber (38.1 cm I.D.) resulted in a water velocity of 0.375 cm per second in the direction of the interface drain. Striped bass oriented into this current and usually remained in the side of the chamber where they were introduced. Overhead "cool white" fluorescent lighting provided 2000 lux of uniform illumination over the avoidance apparatus. Nylon plankton netting (250 µm mesh) was placed over each end of the avoidance chamber to prevent movement of striped bass larvae into the mixing boxes. Immediately after fish were placed in the chamber, a black plastic shield was erected around the apparatus to prevent disturbance from outside.

Striped bass were acclimated in the apparatus for 30 minutes. Five counts of the number of fish on each side of the interface drain were then taken at 5-minute intervals. Immediately after the fifth count, chlorine was added by syringe pump to one side of the apparatus. A second series of five counts were then conducted at 5-minute intervals to determine the number of fish on each side of the interface drain. Total residual chlorination concentrations in the water were measured immediately after each avoidance test. Water samples were taken from sample tubes in the fish-entrance; chlorine-sampling ports.

The avoidance apparatus was emptied and flushed repeatedly with large volumes of water for 15 to 20 minutes after each test. Tests were then repeated (using new fish) with chlorine added to the alternate end of the avoidance apparatus.

Numbers of fish observed on respective sides of the interface drain for each of the five pre-chlorine exposure (control) intervals were subjected to individual chi-square (X^2) analysis (assuming a 1:1 ratio for the two classes) and the individual X^2 values were summed. A pooled chi-square analysis was also conducted on the sum of the fish observed on each side of the interface. The X^2 values for each of these tests were then computed for heterogeneity (Snedecor 1956; Sokal and Rholf 1969). Similar analyses were conducted for the second series of five counts made after chlorine infusion began.

These analyses were designed to determine: (1) differences in the observed and expected ratio for the sum of individual and pooled observations prior to addition of chlorine; (2) differences in observed and expected ratio for the sum of individual and pooled observations after chlorine addition; (3) heterogeneity between the sum of the individual observations and the pooled observations prior to and after addition of chlorine.

Chemical Analysis

Total residual chlorination (TRC) concentrations (free + combined chlorine) were measured with a Wallace and Tiernan Amperometric Titrator. Phenylarsine oxide and pH buffers used were standard Wallace and Tiernan reagents. The 5 percent KI solution and NaOCl (Mallinckrodt, Analytical Reagent, minimum 5.0 percent chlorine) stock solutions were prepared in chlorine demand-free water.

Stock solutions for all biological tests and chemical analyses were maintained in brown glass bottles at 4.0 C prior to use.

Total residual chlorination determinations were conducted using procedures outlined in Standard Methods (American Public Health Association 1971). Particular emphasis was given to sample handling and the time required to perform titrations. All samples were taken directly from the hatchery jars, treatment aquaria, or avoidance appa-

ratus and titrated to an end point within two minutes. Agitation of samples was kept to a minimum to reduce the potential for error caused by "flashing-off" or photodecomposition of residual chlorination (Environmental Protection Agency 1974).

Nominal amounts of chlorine added (mg/l) as NaOCl and measured ranges and means for TRC (mg/l) detected in each series of tests are reported with the test results. Characteristics of the test water used during this study are reviewed in Table 1.

Results

Developing Embryo Tests

The nominal and measured TRC concentrations for bioassays with developing striped bass embryos are given in Table 2. Exposure of approximately 8,500 embryos to each respective test concentration of TRC was begun 8 to 9 hours after fertilization.

No larvae emerged from embryos exposed to a TRC concentration of 0.31 mg/l (Fig 3). Determination of the stage at which development ceased was difficult because of rupture of the yolk sac and escape of the yolk into the chorionic cavity.

Only 3.5 percent of the embryos exposed to 0.07 mg/l TRC hatched. Many of the larvae had a curvature in the vertebral column which occurred 3 to 7 somites posterior to the oil globule. The curvature was generally downward. This vertebral column deformity or inability of the larvae to straighten their bodies suggests a possible cause for poor emergence, since many embryos developed to a stage just prior to emergence (Hayless 1972), but failed to hatch. Larvae which did hatch exhibited a very weak swimming response; they were observed on the bottom of the holding aquarium, moving only a few millimeters into the water column in attempts to swim to the surface.

Twenty-three percent of the eggs exposed to 0.01 mg/l TRC hatched. Many larvae at this exposure concentration had difficulty detaching themselves from the chorion as they hatched.

Survival of developing eggs and emergence of larvae were similar for the lowest exposure concentration of TRC < 0.01 (Se

TABLE 1. Characteristics of water used in tests with the striped bass, *Morone saxatilis*.

Characteristic measured	Concentration mg/l
Total Alkalinity ($CaCO_3$)	22
Carbonate Alkalinity ($CaCO_3$)	0.00
Bicarbonate Alkalinity ($CaCO_3$)	22
Hardness ($CaCO_3$)	460
Silica (SiO_2)	1.0
Calcium	35.3
Magnesium	90
Bicarbonate (HCO_3)	27
Sulfate (SO_4)	170
pH	6.7-6.9

TABLE 2. Nominal amounts of chlorine added (mg/l) as NaOCl and measured concentrations of TRC (mg/l) for tests with developing striped bass eggs. The percentage recovery is based on the mean TRC concentration detected at respective exposure concentrations.

Nominal TRC mg/l	Measured TRC mg/l: Range	Measured TRC mg/l: $\bar{x}$	Percent Recovery
1.0	0.19-0.22	0.21	21.0
0.50	0.06-0.08	0.07	14.0
0.25	0.01-0.01	0.01	4.0
0.10	<0.01[1]	<0.01[1]	-

[1] Below detection limit with amperometric titration method used during this study.

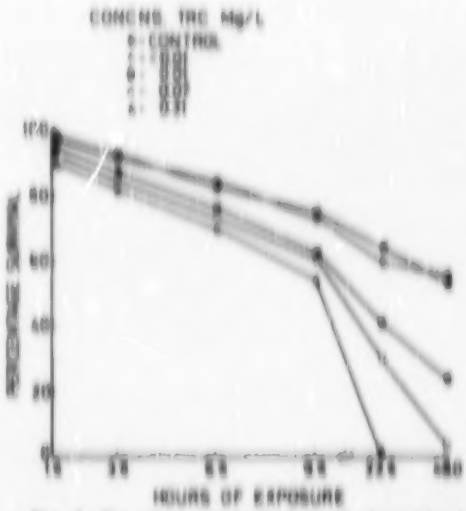

Fig. 3. Percentage survival of developing striped bass eggs exposed to TRC in flowing brackish water (1.0-3.0 ppt salinity). Time intervals are hours of exposure. Eggs were 8 to 9 hours old when the test began.

percent emergence) and for controls (83 percent emergence; Fig. 3). These larvae showed a strong swimming response in their respective holding aquaria. They moved to the surface in a burst or rapid swimming and slowly sank back to the bottom with their yolk sac oriented toward the water surface.

Incipient LC50 Bioassays

Nominal and measured TRC concentrations for bioassays with the three age groups of larvae used in the incipient LC50 bioassays are given in Table 3.

Toxicity tests with 2-day-old prolarvae and 30-day-old juveniles indicate similar sensitivity for these two age classes. Twelve-day old-larvae were less sensitive (Fig 4).

Regression analysis showed that the three toxicity curves fit the power equation ($Y = AX^b$). The correlation coefficients for the tests were: 2-day-old prolarvae, 0.88; 12-day-old larvae, 0.92; and 30-day-old juveniles, 0.76.

The incipient LC50 for each age group was estimated by determining the portion of the toxicity curve asymptotic to the ordinate and by reading the corresponding TRC concentration from the abscissa below. The incipient LC50 was: 0.04 mg/l TRC for 2-day-old prolarvae, 0.07 mg/l for 12-day-old larvae, and 0.04 mg/l for 30-day-old juveniles.

Two-day-old prolarvae died rapidly at the two highest exposure concentrations of TRC, 1.4 and 0.80 mg/l. Prior to death, they were observed swimming in very tight concentric circles and darting from the bottom to the surface of the treatment aquaria. When deaths occurred at the lower exposure concentrations, 0.29 to 0.17 mg/l TRC, prolarvae gradually became less active, rather than acutely distressed. They were observed swimming to the surface and

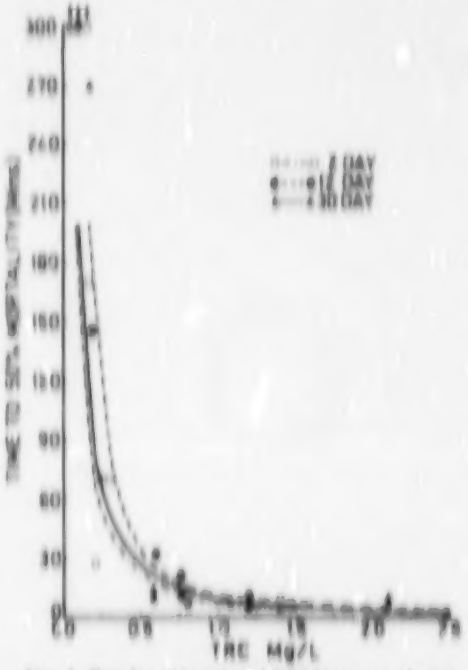

Fig. 4. Results of incipient LC50 bioassays. Incipient LC50 = 0.04 mg/l TRC for 2-day-old prolarvae, 0.07 mg/l for 12-day-old larvae, and 0.04 mg/l for 30-day-old juveniles. Data points with arrows ↑ are for TRC concentrations of 0.08–0.03 mg/l. No deaths were observed at these concentrations after 48 hours of exposure.

TABLE 3. Nominal amounts of chlorine added (mg/l) as NaOCl and measured concentrations of TRC (mg/l) for incipient LC50 bioassays with three age groups of striped bass larvae. Percentage recovery is based on the mean TRC concentration detected at respective treatment levels.

	Measured TRC Concentration (mg/l)								
	2 day old prolarvae			12 day old larvae			30 day old juveniles		
Nominal TRC Concentration (mg/l)	range	$\bar{X}$	percent recovery	range	$\bar{X}$	percent recovery	range	$\bar{X}$	percent recovery
10.0	–	–	–	–	–	–	1.93–2.36	2.14	21.5
5.6	1.39–1.42	1.40	25.0	0.85–1.33	1.07	19.1	0.56–0.59	0.58	10.4
3.2	0.69–0.92	0.80	25.0	0.71–0.81	0.76	23.8	0.21–0.24	0.23	7.1
1.8	0.27–0.30	0.29	16.1	0.57–0.61	0.59	32.8	0.14–0.19	0.17	9.4
1.4	0.19–0.21	0.20	14.2	–	–	–	–	–	–
1.0	0.16–0.17	0.17	17.0	0.17–0.22	0.20	20.0	0.07–0.08	0.08	8.0
0.56	0.08–0.09	0.09	16.1	0.09–0.10	0.10	17.8	0.03–0.04	0.04	7.0
0.32	0.02–0.03	0.03	9.4	0.02–0.03	0.03	9.4	0.02–0.03	0.03	6.3

slowly sinking toward the bottom with the yolk sac oriented toward the water surface. Just prior to death, the prolarvae lay on the bottom of treatment aquaria and exhibited very weak body tremors.

Twelve-day and 30-day-old larvae showed similar behavioral responses to exposure concentrations of TRC in which deaths were observed.

Histopathology

Tissues of control fish appeared normal with good fixation, except for an infrequent gill and pseudobranch artifact (Fig. 5). This artifact consisted of edematous separation of gill and pseudobranch squamous epithelium and pilaster (pillar) cells from the capillary plexus (Fig. 5, arrow). This artifact is not to be confused with degenerative membrane alterations in gill and pseudobranch of chlorine-exposed fish as described below.

Fig. 6 shows normal gill lamellae and branchia from a control fish. Note the close relationship of lamellar capillaries and overlying squamous, respiratory epithelium.

Figs. 7 and 8 show gills of fish exposed to 2.36 mg/l TRC for 7 minutes and sacrificed alive. The following degenerative changes are apparent: separation of squamous respiratory epithelium from pilaster and capillary tissues; general sloughing of pilaster cells in gill lamellae; breakdown of squamous cell epithelium and sloughing of gill tissues.

Fig. 9 shows normal pseudobranch structure in a control fish. In pseudobranch tissues of fish exposed to 2.36 mg/l TRC, the major degenerative change was the extreme separation of squamous epithelium from underlying pilaster cells or endocrine cells (Fig. 10). Frequency of these alterations was 100 percent for fish exposed to 2.36 mg/l TRC. Lesions were also found consistently in fish exposed to TRC concentrations as low as 0.21 mg/l and sacrificed alive after 71 minutes of exposure. None of the control fish or those from concentrations of 0.17 to 0.02 mg/l TRC consistently displayed these lesions.

Avoidance Tests

The nominal amounts of chlorine added and measured TRC concentrations for replicate avoidance tests are given in Table 4.

Twenty-four-day-old striped bass larvae showed statistically significant and reproducible avoidance responses to measured TRC concentrations of 0.79–0.82 and 0.39–0.33 mg/l. Larvae did not avoid 0.16–0.18 mg/l TRC (Table 5).

Differences in the total and pooled X^2 values were not significant in any tests (Table 5). The five control counts indicate equal distribution of striped bass larvae on each side of the interface drain (with little movement across the interface) prior to the addition of chlorine. After chlorine exposure began, lack of positional heterogeneity showed that movements of larval striped bass were uniformly in one direction, away from the side of the avoidance apparatus where chlorine was introduced.

Chemical Analysis

Measured TRC concentrations (mg/l) showed considerable variation from specific nominal (calculated) TRC dosing rates in flowing water tests. For example, in Table 3, we compared the measured TRC concentrations (mg/l) for incipient LC50 tests with 3-, 12- and 30-day-old fish. An increase is evident in the "chlorine demand" of the test water during the bioassay with 30-day-old juveniles. This increase in "chlorine demand" is obvious for all of the measured TRC concentrations and resulted in a decrease in the percentage recovery of TRC for respective nominal dosing rates.

The test with 30-day-old juveniles was begun on 28 April 1975. During the test, we observed a phytoplankton bloom in the brackish water pond from which test water was pumped. The increase in "chlorine demand" may have resulted from chlorine degradation in the oxidation of phytoplankton cells or oxidation of dissolved organics associated with the phytoplankton bloom.

Discussion

Our data show an obvious decrease in the percentage emergence of striped bass eggs at a measured TRC concentration of 0.01 mg/l.

In a study of the toxicity of chlorine to developing eggs and young larvae of the plaice, *Pleuronectes platessa*, Alderdice (1972) learned that the embryos apparently

Figs. 5 -10. 5. Edematous artifact, control fish pseudobranch. 6. Normal gill lamellae and branchia from a control fish. 7-8. Gills of fish exposed to 2.36 mg/l TRC for 7 minutes and sacrificed alive. 9. Normal control fish pseudobranch. 10. Pseudobranch tissues of fish exposed to 2.36 mg/l TRC and sacrificed alive.

7

8

were more resistant to chlorine toxicity than the larvae. The 75-hour LC50 was 0.64 mg/l TRC for embryos exposed beginning 4 days after fertilization. In contrast, the 192 hour LC50 was 0.11 mg/l TRC for embryos whose exposure to chlorine began 5 days after fertilization. In tests with striped bass, we observed that embryo mortalities for all of the measurable TRC concentrations (0.31–0.01 mg/l) were similar for the first 9.5 hours of exposure. During the time interval from 9.5 to 23.5 hours, the number of mortalities increased markedly. These embryos, 17.5 to 41.5 hours after fertilization, were progressing through the late gastrula and neurula stages of development (Bayless 1972).

The data reported by Alderson (1972) suggest that plaice embryos are probably more resistant to TRC than striped bass embryos. The greater sensitivity of developing striped bass embryos to TRC in contrast to plaice embryos may be related to several factors. Specific differences in the sensitivity of the embryos of each species to chlorine may be related to the thickness of the chorion or other morphological characteristics. In addition, our exposures were continuous beginning 8 to 9 hours after fertilization until the time of hatching, whereas Alderson (1972) used tests of 72 to 192 hours duration to estimate LC50 concentrations of TRC for plaice embryos.

Since our bioassay used only a single group of embryos with no replication of exposure concentrations, additional tests should be conducted to confirm our results, preferably with exposures beginning at the time of egg fertilization or immediately prior to fertilization.

Results of incipient LC50 bioassays with three age groups of larval striped bass indicated that 2-day-old larvae and 30-day-old juveniles were more sensitive than 12-day-old larvae. Alderson (1974) reported a decrease in sensitivity with increased age for larval plaice, *Pleuronectes platessa*, and Dover sole, *Solea solea*. This trend was detected in our tests with 2-day-old prolarvae and 12-day-old larvae. However, 30-day-old striped bass juveniles showed a response similar to 2-day-old prolarvae.

Histological examination of surviving 30-day-old juveniles indicated that gill and pseudobranch damage occurred in individuals exposed to TRC concentrations as low as 0.31 mg/l for 71 minutes. Servizi and Martens (1974) reported similar gill damage

TABLE 4. Nominal amounts of chlorine added (mg/l) as $Ca(OCl)_2$ and measured TRC concentrations (mg/l) for replicate avoidance tests. The percentage recovery is based on the mean TRC concentration detected at respective exposure concentrations.

Nominal conc. chlorine added (mg/l as $Ca(OCl)_2$)	Measured TRC conc. (mg/l)			
	Replicate A	Test B	S	Percent Recovery
1.0	0.79	0.64	0.51	14.5
0.5	0.29	0.32	0.31	9.9
0.2	0.15	0.18	0.17	9.4

TABLE 5. Summary of data for avoidance tests conducted with 14 day old larval striped bass. X^2 values are given for replicate tests at each exposure concentration of total residual chlorination (TRC). Tests were conducted at 18 ± 1°C.

Replicate Test	TRC mg/l	df	Total X^2	df	Pooled X^2	df	Heterog. of Samples Total X^2 – Pooled X^2
A	Control	5	0.40	1	0.16	6	0.24
	0.79	5	34.60***	1	34.44***	6	0.96
B	Control	6	2.80	1	2.56	6	0.24
	0.82	5	23.20***	1	23.04***	6	0.16
A	Control	5	2.80	1	2.56	6	0.24
	0.29	5	20.60***	1	14.44***	6	6.16
B	Control	5	0.40	1	0.16	6	0.24
	0.31	5	11.40*	1	5.76*	5	5.64
A	Control	5	3.40	1	3.24	6	0.16
	0.15	5	1.60	1	1.44	6	0.16
B	Control	5	1.00	1	1.00	6	0.00
	0.18	5	0.80	1	0.64	6	0.16

* $p = 0.05$
*** $p = 0.005$

in fingerling sockeye salmon, *Oncorhynchus nerka*, exposed to chlorinated sewage effluents (0.22 mg/l TRC) for 1 to 3 hours. Damage in the salmon included hyperplasia, swollen epithelial cells, and separation of the epithelium from pillar cells.

In a study with juvenile spot, *Leiostomus xanthurus*, (Middaugh et al. 1977), we found pseudobranch and gill damage in individuals exposed to 1.57 mg/l TRC for 95 minutes and sacrificed alive. The pseudobranch lamellar epithelial membranes were distorted and separated from other tissues. The edematous separation of epithelial membranes presented a scalloped border to the pseudobranch. The gill filament and lamellar organization was also disrupted by severe epithelium damage. Complete separation of squamous respiratory epithelium and capillary tissues occurred in the gill lamellae. Alderson (1974) observed evidence of slight gill damage in Dover sole, *Solea solea*, exposed to 0.068 mg/l total residual chlorine for 20 days; fish exposed to concentrations of 0.036 mg/l TRC or less did not reveal any differences in comparison to unexposed (control) fish.

Loss of integrity in structure of gills and pseudobranches of fishes would probably be reflected in dysfunction in respiration, osmoregulation, and oxygen utilization. The latter function may relate to the suggested endocrine function and oxygen secretion capabilities of the pseudobranch in teleosts (Ashley 1975). Gill diffusion control and respiration would presumably be lost in an organ that was structurally disorganized.

Results reported by Grothe and Eaton (1975) suggest that chloramine-induced death in the fathead minnow, *Pimephales promelas*, was related to methemoglobinemia. They attributed the formation of methemoglobin to chloramine-induced oxidation of red blood cells as they pass through the gills, thus resulting in anoxia.

Our avoidance tests indicated that 24-day-old striped bass larvae will avoid measured TRC concentrations as low as 0.29-0.33 mg/l at 1.0 to 3.0 ppt salinity and 18 ± 1 C. A temperature dependent avoidance response was reported by Meldrim et al. (1974) in tests with white perch, *Morone americana*. At 1.5 ppt salinity and 4 C, avoidance was observed for concentrations of free chlorine ranging from 0.07 to 0.25 mg/l. For tests conducted at 17 to 22 C, free chlorine concentrations of 0.02 to 0.08 mg/l were avoided.

In research with juvenile spot, *Leiostomus xanthurus*, (Middaugh et al. 1977), we also found temperature dependent avoidance responses to TRC. At 19 and 23 ppt salinity and 10 C, a TRC concentration of 0.18 mg/l was necessary to cause avoidance; for tests conducted at 15 and 20 C, TRC concentrations as low as 0.05 mg/l were avoided.

Results of this study indicate that the early life stages of the striped bass are sensitive to TRC in flowing brackish water. Striped bass eggs spawned or transported by water currents into areas receiving chlorinated effluents may be particularly susceptible. Young larvae and juvenile striped bass were also sensitive to TRC. Toxicological and avoidance tests with these life stages showed that chlorine could have a direct toxic effect or may eliminate habitat because of an avoidance response.

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Behavioral Avoidance Responses of Estuarine Fishes to Chlorine

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Introduction

The amount of chlorine released into estuarine and marine waters has caused concern regarding its environmental impact. Although toxicity tests reveal what levels can be tolerated, sublethal effects are also important. This paper is concerned with behavioral avoidance, a major sublethal effect. Considerable data have been accumulated on several fishes and macroinvertebrates. The results of tests with white perch, *Morone americana*, and Atlantic silverside, *Menidia menidia*, are presented because they exemplify avoidance behavior in estuarine waters. However, because the studies are ongoing, the analyses and conclusions drawn are tentative.

Methods

Avoidance responses were determined under varying conditions of temperature, salinity, dissolved oxygen, and light level following the yearly ambient temperature and salinity cycles of the Delaware River estuary. Tests were conducted using the modified Shelford-Allee apparatus, a divided trough further divided into quadrants. The apparatus and experimental design is described in detail by Meldrim, Gift, and Petrosky (1974). Experimental chlorine concentrations were achieved using a solution of calcium hypochlorite and were measured using a Fischer and Porter amperometric titrator. However, since the chemistry of chlorine in estuarine waters is complex and the ratios of halogens in these tests unknown, concentrations are expressed as residual oxidant. Salinities used in testing were between 0 and 7 ppt. Because the Atlantic silverside is generally not found in low salinity waters, no tests were conducted on that species at salinities less than 2.5 ppt. Both white perch and Atlantic silverside are present in the Delaware River estuary throughout most of the year and testing was conducted over the annual range of ambient water temperatures.

Responses to residual oxidant were tested under the following conditions: (1) near saturation oxygen levels and ambient temperatures in all quadrants, (2) near saturation oxygen levels in all quadrants with ambient temperatures in the control quadrants (which contained no oxidant) and ambient temperatures plus approximately 2 to 3 C in the experimental quadrants (which contained chlorine dose), (3) near saturation oxygen levels in the control quadrants and levels approximately 3 ppm below saturation (which is about 70–80% saturation) in the experimental quadrants with ambient temperatures in all quadrants, and (4) near saturation oxygen levels with ambient temperatures in the control quadrants and oxygen levels approximately 3 ppm below saturation with ambient temperatures plus approximately 2 to 3 C in the experimental quadrants. These conditions were tested at both high (1076 lux) and low (430 lux) light levels.

Results and Discussion

The time of exposure to test concentrations of oxidant in these studies was relatively short. Under this experimental regime the initial (and lowest concentrations) usually elicited preference, particulary in tests in which a ΔT accompanied the experimental concentration. As the oxidant concentra-